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Grafting of Polypeptides on Solid Substrates by Initiation of *N*-Carboxyanhydride Polymerization by Amino-Terminated Self-Assembled Monolayers

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A series of mixed self-assembled monolayers of functionalized ($Br(CH_2)_{11}SiCl_3$) and unfunctionalized ($CH_3(CH_2)_{10}SiCl_3$) alkyltrichlorosilanes of different compositions have been prepared on bulk silicon substrates. By *in situ* modification of these monolayers the bromo end groups were transformed to amino end groups as shown by X-ray photoelectron spectroscopy measurements. The change of hydrophilicity was monitored by water contact angle measurements, showing the expected decrease of contact angles with increase amino group content on the surface. These substrates were used to initiate *N*-carboxyanhydrides in dioxane to yield α-helical polypeptides grafted from the surface. The thicknesses of the obtained polymer layers were measured with ellipsometry and X-ray reflectometry. Fourier transform infrared measurements confirm that the grafted polymers are in the α-helical conformation.

Introduction

The tethering of polymers to surfaces has gained increasing interest in recent years. The motivation for this arises not only from fundamental research but also for practical reasons. The combination of the properties of a supporting material and a polymer or rather the fixation of the polymer properties on a substrate is promising for a wide variety of possible applications.[1] Due to the large number of polymer systems and substrate materials available, the field of possible applications reaches from covalent tethering of polystyrene[1,2] to adhesive tethering of block copolymers.[3]

The tethering of rigid rodlike synthetic polypeptides like poly(benzyl L-glutamate) (PBLG) to silicon is of special interest, as manifested by several publications.[4−8] These rigid rodlike macromolecules differ from polymers with flexible secondary structure resulting in some unique characteristics. For example, PBLG, due to its anisotropic molecular shape arising from its α-helical structure, shows a large dipole moment of 8000 D along the molecular axis, as well as a high hyperpolarization β of 5×10^{-28} esu for $M_n = 500\,000$ g/mol.[9] Therefore, it is a promising material for application as a novel orienting layer in liquid crystal displays.[8] Furthermore, a nonlinear optical response like second harmonic generation (SHG) can be expected if a monomolecular polyglutamate film has an unidirectional orientation of the polymer backbones.[10] An approach to such an unidirectional orientation of the polypeptide helices is covalent attachment of one of the end groups to the substrate with a high grafting density (Figure 1) as first described by Sano *et al.*[5] and named *chemical reaction alignment* (CRA).

There are two ways to achieve grafting: (1) initiation of the monomer *N*-carboxyanhydride (NCA) polymerization by the surface (grafting from);[6,7] (2) coupling of preformed polymers to the surface (grafting onto).[4,5] Regarding the initiation from the surface, one must consider that two mechanisms of NCA polymerization are competing depending on the initiator used. The activated monomer (a.m. mechanism is initiated by bases and the

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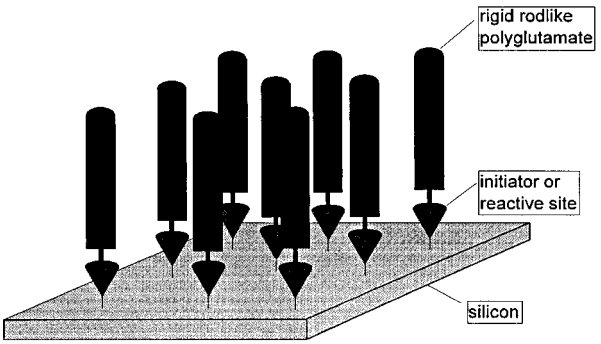

Figure 1. Schematic of an aligned polyglutamate monolayer.

amine or protic mechanism initiated by primary amines.[11] Exclusively with the latter the initiator is inserted into the growing polymer chain. Therefore, it is necessary to establish primary amino groups on the surface to graft a polymer from the surface. On the other hand primary amines can also act as a base during initiation leading to nongrafted polymer by a change of mechanism. The greater the steric hindrance of the primary amines sites on the surface, the more the undesired a.m. mechanism is preferred. Taking into account that a polymerization from a surface runs under demanding steric conditions, it is obvious that the initiator layer should have a low surface roughness and a distribution of initiator sites as perfect as possible. The coupling of preformed polymers again requires reactive sites either on the surface or at the polymer terminus which can couple to certain groups of the other component.

The grafting of polyglutamates from a surface has been reported for a case using spherical substrates (Aerosil A200V,[12] carbon black[13]) modified with (aminopropyl)-trimethoxysilane (APS) as initiator on the surface. Although the tethering onto flat substrates presents an even greater challenge because of the lower surface area, resulting in a smaller amount of initiator or reactive sites, it has been carried out by different groups following one of the two ways mentioned above. Enriques *et al.*,[4] for example, used a self-assembling technique of PBLG containing a disulfide moiety attached at its N-terminus onto gold (grafting onto). Coupling of PBLG to silicon modified with (*N*-(2-aminoethyl)-3-aminopropyl)methyldimethoxysilane was done by Sano *et al.*[5] with dicyclohexylcarbodiimide. Whitesell et al. first described a "*grafting from*" procedure for polypeptides on flat surfaces.[6a] They used indium−tin oxide modified with APS or a special designed initiator on a gold surface to initiate a NCA polymerization. The latter initiator was designed to fit the surface requirements of the polypeptide helix and therefore reduce steric hindrances.[6a] Wieringa and Schouten grafted PBLG and poly(methyl L-glutamate) from silicon modified with APS[14,15] or (4-aminobutyl)-dimethylmethoxysilane[7,15] using a solution or a melt of the monomers, respectively.

In the present paper we describe our results from polymerization of benzyl L-glutamate−NCA (Figure 2) using mixed self-assembled monolayers (SAMs[16]) with various concentrations of amino end groups. In contrast to APS,[17] long chain alkyltrichlorosilanes give defined and

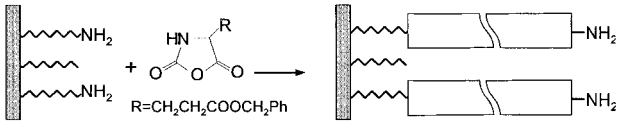

Figure 2. Polymerization of γ-benzyl L-glutamic acid−NCA from an amino-terminated surface.

reproducible monolayers.[18] While thiols form monolayers on gold due to the exclusive reaction of the thiol group with the gold atoms, silanes form mainly a two-dimensional network at the surface which is tethered to the substrate by some covalent bonds.[19] Nevertheless the monolayers formed by silanes are very robust.

The alkyl chain spacer provides stabilization of the molecular assembly through interchain van der Waals interaction. The order within the monolayer can, therefore, be adjusted to some extent by the length of the alkyl chain or the temperature used for the silanization process.[20] To produce monolayers with active sites, terminal functional groups have to be present in the silanization agent. These terminal groups must not compete with the surface active headgroup for adsorption to the surface or react with it. For that reason amino and trichlorosilyl groups are not compatible. Amino-terminated long alkyl chain trimethoxysilanes are stable but give disordered layers due to the chemical interactions of the amino groups with the substrate.[21] Nevertheless, following Balachander and Sukenik amino-terminated monolayers can be obtained by deposition of bromo-terminated alkyltrichlorosilane and subsequent *in situ* modification on the surface.[22] In order to reduce the steric hindrances for the polymerization reaction, it is favorable to adjust the density of initiating groups at the surface. According to Whitesell et al. this can be done by use of special designed initiator molecules.[6] We choose a more facile way to accomplish this: By preparation of mixed monolayers from functionalized (Br end group) and nonfunctionalized (CH_3−end group) undecyltrichlorosilane in different ratios, the amino groups can be diluted at the surface. To the best of our knowledge mixed silane monolayers of this kind have not been previously described.[23] Frank and co-workers reported mixed, amino-terminated SAMs prepared by backfilling (preparation of the SAM in two steps).[24]

Experimental Section

Materials. Unless otherwise stated all reagents and chemicals were obtained commercially. Dioxane was distilled from sodium. Hexadecane (Aldrich) was purified by percolation twice

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(23) Very recently mixed monolayers prepared by coadsorption of hexadecyltrichlorosilane and (bromoheptadecyl)trichlorosilane have been reported: Fryxell, G. E.; Rieke, P. C.; Wood, L.; Engelhard, M. H.; Williford, R. E.; Graff, G. L.; Campbell, A. A.; Wiacek, R. J.; Lee, L.; Halverson, A. *Langmuir* **1996**, *12*, 5064.
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through neutral alumina (ICN, 32-63) and used immediately. Triethylamine (Fluka) was distilled from calcium hydride. Acetone (Riedel-de Haën, 99.5%), dichloromethane (Fluka), 2-propanol (Fluka), 1-undecene (Aldrich), undecyl-10-enyl bromide (Lancaster), trichlorosilane (Aldrich), hydrogen hexachloroplatinate(VI) hexahydrate (Johnson Matthey), sodium azide (Fluka), lithium aluminum hydride (1 M in THF, Aldrich), benzyl alcohol (Acros), and glutamic acid (Fluka) were used as received.

Surfactant Synthesis. *1-Bromo-11-(trichlorosilyl)undecane.* Undecyl-10-enyl bromide (12.5 g, 60 mmol) was added to an argon-purged round bottom flask containing approximately 5 mg of hydrogen hexachloroplatinate(VI) hexahydrate in 0.1 mL 2-propanol. A 2−3 mol excess of trichlorosilane (10 mL, 150 mmol) was added with stirring. The flask was fitted with a water-cooled condenser, and the mixture was refluxed for 2 h under argon. Excess trichlorosilane was removed by short-path distillation. The residue was purified by Kugelrohr distillation, using a commercially available apparatus (BÜCHI GKR-51). The distillation was carried out at 0.01−0.02 mbar and ca. 120−130 °C.[25] ^{1}H NMR (80 MHz, $CDCl_3$) δ (ppm): 1.0−1.65 (m, 18H), 1.70−2.0 (m, 2H), 3.3−3.5 (t, 2H).

1-Trichlorosilylundecane. 1-Undecene was converted into 1-trichlorosilylundecane by using the above hydrosilylation procedure and isolation by Kugelrohr distillation, using a commercially available apparatus (BÜCHI GKR-51). The distillation was carried out at 0.01−0.02 mbar and ca. 100 °C.[25] ^{1}H NMR (80 MHz, $CDCl_3$) δ (ppm): 0.7−1.0 (t, 3H), 1.1−1.6 (m, 20H).

Substrate Preparation. Silicon wafers (Wacker Chemitronics, both sides polished) with a native oxide layer of 15−16 Å thickness were cut to measure 2.5 cm × 6 cm. Substrates were wiped with an acetone-soaked cotton swab, successively sonicated in soap water, rinsed with large amounts of distilled water, and sonicated in dichloromethane. Substrates were then placed in freshly prepared "piranha" solution (70/30 (v/v), concentrated sulfuric acid, 30% hydrogen peroxide) for 1 h at 60−80 °C. *Caution: Piranha solution is extremely corrosive, can react violently with organic compounds, and should not be stored for any length of time.*[26] Subsequently substrates were rinsed with large amounts of distilled water, dried under an argon stream, and used immediately.

Silanization. Mixed monolayers were prepared by immersing clean, dry silicon substrates under argon into a hexadecane solution (0.3%) of freshly Kugelrohr distilled 1-bromo-11-(trichlorosilyl)undecane and 1-(trichlorosilyl)undecane in the desired ratio in clean 100 mL vials. The solution was stirred with a glass rod and vials were capped, placed in a sand bath, and heated to 40−50 °C for 4−6 h. Subsequently SAMs were withdrawn from the solution and immersed in CH_2Cl_2. After further rinsing with CH_2Cl_2, the SAMs were wiped with an acetone-soaked cotton swap (wiping in one direction only)[27] and immersed in hot distilled water for a short time.

In Situ Modifications. *Azide-Terminated SAMs.* Bromide-terminated SAMs were placed in a supersaturated solution of NaN_3 in dry DMF (1.5 g on 100 mL). The solution (together with the undissolved NaN_3) was stirred at room temperature. After 24 h the SAMs were rinsed with distilled water and wiped with an acetone-soaked cotton swab.[27]

Amino-Terminated SAMs. The azide-terminated SAMs were placed in lithium aluminum hydride solution (0.2 M in THF). After 24 h the SAMs were soaked in THF for an additional 24 h. SAMs were then placed in 5% HCl solution for 5 h to complete hydrolysis of the aluminum complexes, rinsed with distilled water, wiped with acetone, and placed in triethylamine for 24 h in order to convert the $-NH_3^+$ into $-NH_2$ groups.

Polymerization. Benzyl L-glutamate and benzyl L-glutamate−NCA were synthesized according to Block.[28] Amino-terminated SAMs were placed in a 2−3% solution of γ-benzyl L-glutamate NCA in dry dioxane under argon at room temperature for 14 days. To remove ungrafted material, the wafers were rinsed with THF in a Soxhlet apparatus for a further 3 days.

Ellipsometry. Measurements were carried out with a self-built automatic null ellipsometer at $\lambda = 632.8$ Å and an angle of incidence of 70°.[3b] All calculations were done with a three-layer step-profile model assuming fixed refractive indices of 1.48 for the SAMs[29] and 1.5 for the polymer layers[4] on top of the native SiO_2 layer. This leads to uncertainties of 5−10% in the calculated layer thickness.

Contact Angles. The advancing and receding contact angles of water on the SAMs and polymer films were measured by the tilting stage method (50°) at room temperature using a Krüss G1 contact angle goniometer mounted on a home made tilting base. Humidity was controlled to be 100% by keeping the wells of the goniometer filled with water. Receding and advancing angles were measured on three different drops and results were averaged.

X-ray Reflectometry. X-ray reflectometry (XR) measurements were performed using Cu Kα radiation ($\lambda = 0.154$ nm) from a Rigaku 12 kW rotating anode with a line focus. The reflectometer was designed around Huber goniometers and optical components from Blake Industries, including a graphite primary monochromator to give a constant wavelength resolution of $\Delta\lambda/\lambda = 0.015$. Angular divergence was constant at about 2×10^{-4} radians. All measurements, typically lasting several hours, were performed in air at room temperature. Data sets were corrected for background estimated using measurements with the detector offset from the specular condition.

The XR data were analyzed by assuming a reasonable initial model for the scattering length density profile through the film, calculating the reflectivity curve that such a model would give, and then varying model parameters until acceptable agreement between the experimental and calculated model reflectivity was achieved. The model scattering length density profiles were created by first forming a simple model of four distinct layers, PBLG, SAM, SiO_x, and silicon. Then the interfaces between layers were broadened by convoluting the sharp interface with a Gaussian function to yield diffuse interfaces characterized by the root mean square roughnesses reported in Table 1. Constraints on appropriate values for the scattering length density and roughness parameters for the Si, SiO_x, and SAM are well-known from other work in this research group.[22c] Therefore, these parameters can only vary within narrow limits. The primary parameters for "fitting" the data are those associated with the PBLG layer.

Fourier Transform IR (FT-IR). FT-IR spectra were recorded on a Mattson Galaxy 6020 spectrometer in transmission mode with a pure silicon wafer as a reference. The sample chamber was purged with dry nitrogen before and during measurements. The resolution was set at 4 cm^{-1} and 256 scans were recorded.

X-ray Photoelectron Spectroscopy (XPS). The XPS measurements were performed with a X-Probe 300 (Surface Science Instruments) instrument using monochromatic Al Kα radiation with an energy of 1486.6 eV. Each sample was measured with an experimental resolution of 1.8 eV and a take-off angle of 45°. Charge correction was calculated from the difference of the observed C(1s) binding energy to the calculated value of 284.8 eV. The N(1s) peak was assigned according to ref 30. The compositions were obtained by fitting the peak area using a Gauss function after subtraction of the background. The atomic percentages for the SAM were obtained by correcting the data using XPS sensitivity factors given by the instruments manufacturer (C, 1.0000; N, 1.6783; O, 2.4935).

(25) Because the temperature adjusted at the Kugelrohr apparatus does not correspond to boiling point, exact data cannot be given.

(26) Dobb, D. A.; Bergman, R. G.; Theopold, K. H. *Chem. Eng. News* **1990**, *68* (17), 2.

(27) The wiping was performed to remove some spots which appeared where drops of the solvent, used for rinsing, had evaporated. Most probably these spots arise from material present in the solution, such as siloxane polymers resulting from solution polycondensation. In the case of the azide formation wiping was also necessary to remove sodium azide, which is present as a solid in the supersaturated solution.

(28) Block, H. *Poly(γ-benzyl-glutamate) and other glutamic acid containing polymers*; Gordon and Breach Science Publishers: New York, 1983.

(29) According to Wassermann, S. R.; Tao, Y. T.; Whitesides, G. M. *Langmuir* **1989**, *5*, 1074, who give a refractive index of 1.45 for the SAMs. Altering this value by 0.05 results in less than 1 Å change in calculated thickness.

(30) Moulder, J. F.; Stickle, W. F.; Sobol, P. E.; Bomben, K. D. *Handbook of X-Ray Photoelectron Spectroscopy*; Perkin-Elmer: Eden Prairie, MN, 1992.

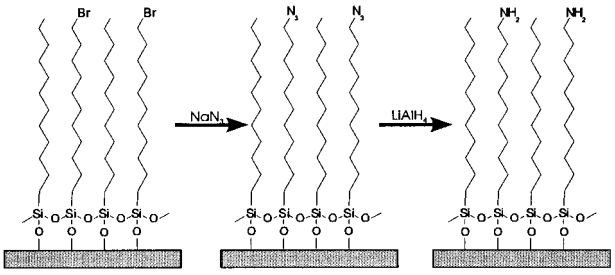

Figure 3. Schematic of the *in situ* modification of mixed SAMs.

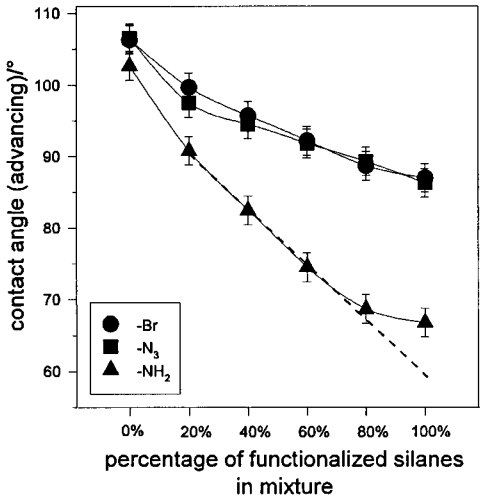

Figure 4. The advancing contact angle (water) on SAMs decrease as the fraction of functional groups increases. The percentage indicated corresponds to the mixing ratio of the silylating agents in solution during the preparation.

Results and Discussion

Mixed SAMs. To obtain monolayers with amino end groups in different amounts, 1-bromo-11-(trichlorosilyl)-undecane and 1-(trichlorosilyl)undecane were mixed in the silanization solution in varying proportions. For the experiments reported here the C11-silanes were chosen in order to permit the terminal functional groups to have sufficient freedom to react in a sterically unhindered fashion but still to have enough interaction energy between the chains to ensure that the molecules are strongly absorbed.[20,31] For the same reason a high-temperature method for the silanization was chosen, which results in more disordered layers.[20b] By *in situ* modification of the surface according to the method of Balachander and Sukenik[22] the bromo end group was displaced by azide. The azide was reduced with lithium aluminum hydride to the amino end group (Figure 3).[22] XPS measurements by Balancher and Sukenik[22a] and Lander et al.[22b] have proven that this procedure yields amino groups at the surface. Furthermore, the preparation and modification have been followed by means of X-ray reflectometry, and difficulties in the original protocol have been corrected.[22b,c] The increase in surface polarity encountered with the modification can be followed by measuring the contact angles of water on the surface (Figure 4).[32] Furthermore, the contact angles are decreasing with increasing concentration of functional groups, reflecting the higher hydrophilicity of the surface. This is in particular valid for the $-NH_2$ terminated wafers since amino groups possess a naturally higher hydrophilicity compared to bromide and azide. Values for contact angles range from 107° for the unfunctionalized SAM to 89° for the 100% bromo- and azide-terminated SAMs, respectively, whereas the values for the amino-terminated SAM ($-NH_2$) range from 103° to 68°. To the best of our knowledge there are no literature data for this kind of mixed SAMs. However, the contact angles of the pure amino-terminated and the pure alkyl SAMs are in good agreement with the literature[22,23] (100% $-NH_2$ groups 63° [33]), although a direct comparison of absolute contact angles measured in different laboratories is very tentative. Results depend on the method used and also on the quality of liquid used.[34] In any case it appears from the results that increasing the portion of functional groups in solution increases the portion of functional (polar) groups at the surface. From a linear extrapolation of the measured values for the mixed monolayers, a value of approximately 60° is expected for the 100% amino-terminated monolayer. The difference between the expected (60°) and the measured value (68°) may be due to incomplete *in situ* conversion caused by steric hindrances. On the other hand Bain *et al.* found a strong deviation from linearity in the dependence of the wettability on composition for monolayers obtained by adsorption from mixed solutions of methyl- and hydroxy-terminated thiols onto gold. It was concluded that the polar groups are more hydrophilic when they are in the nonpolar environment provided by methyl groups than when the surface of the monolayer is composed largely of other polar groups.[35] The nonlinear dependence of the contact angles on the composition of the solution can also be explained to be due to a preferential absorption of the bromine-terminated trichlorosilane. Such a preferred absorption in the case of slightly different terminal groups has been found by Whitesides and co-workers for thiol with different terminal groups.[35b,36]

To verify the *in situ* modification of the monolayers XPS measurements are under way. Preliminary results of these investigations are shown in Figure 5. A N(1s) peak is found in the XPS spectra of the amino-terminated SAMs which is not present in the Br-terminated SAMs. The atomic ratio N/C for the SAM after *in situ* conversion increases with increasing amount of Br-terminated silane in the silylation mixture. The nonlinear dependence of the atomic ratio N/C on the composition of the silylating mixture may be an indication of an incomplete conversion of the Br end groups into amino groups or a preferred adsorption of the Br-terminated trichlorosilanes from the silylating mixture. Curve A in Figure 5 approximates how the atomic ratio N/C would change with the silylating mixture composition if the layer deposited at 20% really had 20% NH_2 functionality and complete conversions were achieved for all *in situ* reactions. The discrepancy between curve A and the measured ratios is quite high and this suggests that the alternative explanation may be the dominant one, although no doubt the *in situ* conversions do not have perfect yields. It is possible that Br-terminated silanes are preferentially adsorbed from the deposition mixture as has been found by Whitesides and co-workers for thiols on gold.[35b,36] In this case, if the yields of the in situ conversions were close to 100%, then the measured atomic ratios should lie above a linear behavior, approximated by curve B in Figure 5, expected for nonpref-

(31) Duevel, R. V.; Corn, R. M. *Anal. Chem.* **1992**, *64*, 337.

(32) For better clarity only advancing contact angles are shown. Hysteresis is about 8° for all samples.

(33) The data have to be compared for the $-NH_2$, not for the $-NH_3^+$ species. For the latter the contact angles are normally below 50°.

(34) Silberzan, P.; Leger, L.; Ausserre, D.; Benattar, J. J. *Langmuir* **1991**, *7*, 1647.

(35) (a) Bain, C. D.; Biebyuyck, H. A.; Whitesides, G. M. *Langmuir* **1989**, *5*, 723. (b) Bain, C. D.; Evall, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 7155.

(36) (a) Bain, C. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 6560. (b) Folkers, J. P.; Laibinis, P. E.; Whitesides, G. M. *Langmuir* **1992**, *8*, 1330.

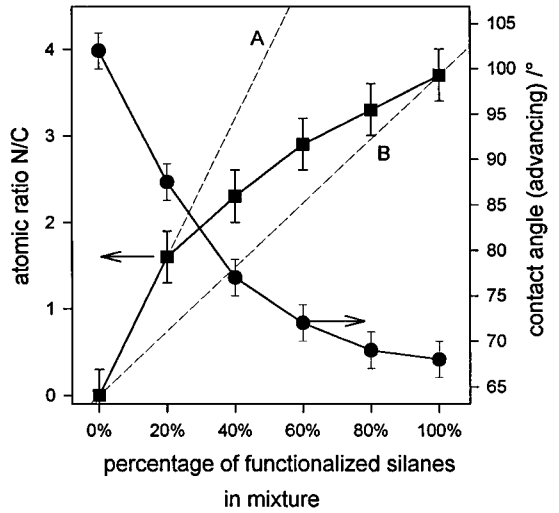

Figure 5. Contact angles and atomic ratio N/C determined by XPS as a function of the composition of the silylating mixture used for SAM deposition. Dotted line A indicates the behavior which would be expected for nonpreferential adsorption and full conversion for all *in situ* reactions. This curve also assumes that full conversion was actually achieved for the SAM deposited from the 20% mixture. Dotted line B illustrates the behavior expected for nonpreferential adsorption if one assumes that the SAM deposited from the 100% mixture was fully converted.

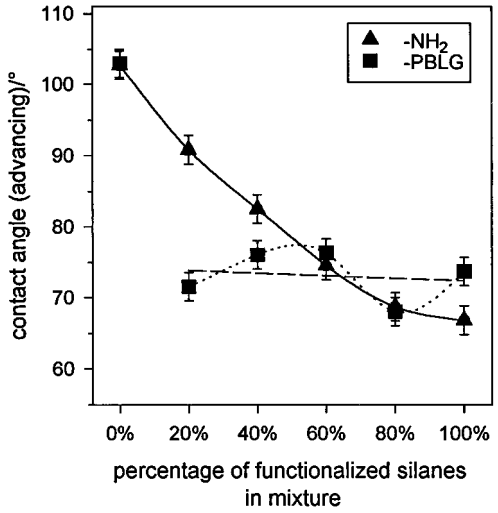

Figure 6. Contact angles before (NH_2) and after (PBLG) polymerization (see text).

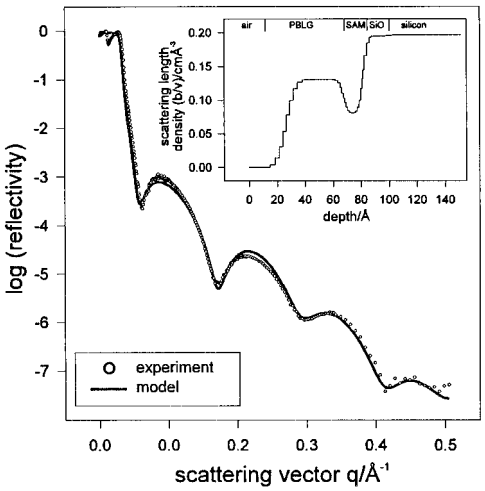

Figure 7. Experimental data and model fit of PBLG polymerized from a wafer with 80% amino groups. The corresponding model scattering length density profile is shown in the insert.

erential adsorption followed by complete conversion. A combination of both mechanisms is possible and cannot be ruled out at present. More detailed XPS measurements are under way to address this. In any case, the present data clearly indicate that the SAMs do have amino functionality after *in situ* conversion and that the amount of this functionality increases substantially, though not linearly, with the fraction of Br-terminated silanes in the silylating mixture.

To verify layer thickness, ellipsometry measurements were carried out, giving values of 19 ± 2 Å for all samples. Taking into account that the calculated thickness for a complete SAM layer with fully extended alkyl chains is 17 Å, the measured values are in strong support of a monolayer coverage.

The preliminary results of X-ray reflectometry on the Br-terminated SAMs are in accordance with a monolayer coverage, too, but also reflect the aforementioned features of silane SAMs. The fitting of the reflectivity curves requires the assumption of an interfacial layer between the SiO_x surface and the alkyl chains as first proposed by Tidswell *et al.*[37] This layer can be ascribed to the two-dimensional polysiloxane network with some adsorbed water. The thickness of this layer is 6.6 ± 1 Å in the case of a 100% Br-terminated SAM. The thickness of the layer of the alkyl chain (with the Br end group) is determined to be 15.6 ± 1 Å, which is in very good agreement with the theoretical value of 15.74 Å. The difference between the thickness of the complete SAM as determined by ellipsometry and from X-ray reflectivity is due to the different sensitivity to the interfacial layer.[37b] Concluding, both X-ray reflectivity and ellipsometry reveal a monolayer coverage.

Polymerizations. Polymerizations were carried out by immersing the wafers in a solution of BLG-NCA in dioxane. After being withdrawn from the polymerization solution and cleaned, the wafers with amino-terminated SAMs have different contact angles, while the contact angles for wafers with CH_3-terminated SAM remained unchanged (Figure 6). This indicates that the change in the contact angles is due to polymer on the surface. The variation of the contact angles as a function of the amino group concentration on the initiating surface is complex. This may be due to different orientations of the peptide chains (see Figure 6, dotted line). If the chains are lying flat on the surface, they expose the nonpolar side groups to the gas phase. If they are standing upright, they orient the more polar amino end groups to the gas phase. However, a deeper discussion of contact angles is difficult since the exact orientation is unknown so far. On the other hand the data also may be interpreted as scattering around a "constant" value, since the differences are just beyond the experimental error of the contact angle determination itself (see Figure 6, broken line).

The thickness of the polymer layer was determined by ellipsometry and X-ray reflectometry (XR) (see Table 1). Figure 7 shows a typical XR measurement with experimental data and fitted curve. The evaluated scattering density profile of the layer is shown in the insert.

Almost all thicknesses obtained by XR are slightly higher than the values from ellipsometry, but in general there is a good agreement. The thickness of the polymer layer ranges from 45 Å for the sample with 20% amino groups to a maximum of 65 Å for the wafer with 40%

(37) (a) Tidswell, I. M.; Ocko, B. M.; Pershan, P. S.; Wasserman, S. R.; Whitesides, G. M.; Axe, J. D. *Phys. Rev. B: Condens. Matter.* **1990**, *41*, 1111. (b) Tidswell, I. M.; Rabedeau, T. A.; Pershan, P. S.; Folkers, J. P.; Baker, M. U.; Whitesides, G. M. *Phys. Rev. B: Condens. Matter.* **1991**, *44*, 10869.

Table 1. Roughness and Thickness of Polymer and Interface Layers

Br-terminated silanes in mixture, %	roughness/Å (±2 Å)			thickness/Å	
	air/ PBLG	PBLG/ SAM	SAM/ SiO	ellipsometry (±5 Å)	XR (±2 Å)
100	16	8	5	46	46
80	12	6	6	50	55
60	16.5	7	6	58	58
40	13	6	5.5	54	65
20	23	7.5	5.7	45	46

amino groups. If one assumes a perpendicular orientation of the polyglutamates, a thickness of 65 Å corresponds to an average degree of polymerization of 42.[38] Whether the initiator concentration of 40% at the surface represents an optimal concentration for the graft polymerization must be confirmed by further investigations. However, also the roughness of this sample is lower than for most of the other samples (Table 1). In particular the 20% sample shows not only the lowest thickness but also the highest roughness. This suggests that at least a certain concentration of initiators on the surface is required. Nevertheless, as long as the orientation of the polymers is unknown, an interpretation of the roughness values with respect to the orientation is speculative.

The conformation of the grafted PBLG can be determined by IR spectroscopy from the position of the amide peaks.[7] Compared to the amine and the ester peaks the position of both amide I and amide II peak is depending on whether the PBLG is in the β-sheet or in the α-helical conformation. For the α-helix conformation the amide I peak is located at about 1650 cm^{-1} and the amide II peak at about 1546 cm^{-1}, whereas for the β-sheet conformation the amide I peak is located at about 1630 and at 1530 cm^{-1} for the amide II peak, respectively.[28] As an example the IR spectrum of the sample polymerized on the surface with 100% amino groups in Figure 8 shows only peaks for PBLG in the helical conformation with no detectable amount of β-sheet material.

Conclusions

Experiments were carried out to clarify whether the grafting of poly(benzyl L-glutamate) from flat surfaces can be improved by adjusting the density of initiator sites at the surface in order to meet the steric requirements of the polymerization reaction. This adjustment was done by simultaneous adsorption of Br- and CH_3-terminated alkyltrichlorosilanes on silicon wafers resulting in mixed monolayers and subsequent *in situ* modification of the bromide into amino groups ($-NH_2$), which are the initiator sites. The formation of monolayers was confirmed by ellipsometry and X-ray reflection. The modification was done by an established procedure and verified by XPS. The increase in surface polarity encountered with this modification was followed by contact angle measurements. In addition, the gradation of amino group concentration at the surface was qualitatively confirmed by contact angle and XPS measurements. However, so far we have no information about the exact concentration and lateral distribution of the initiator sites.

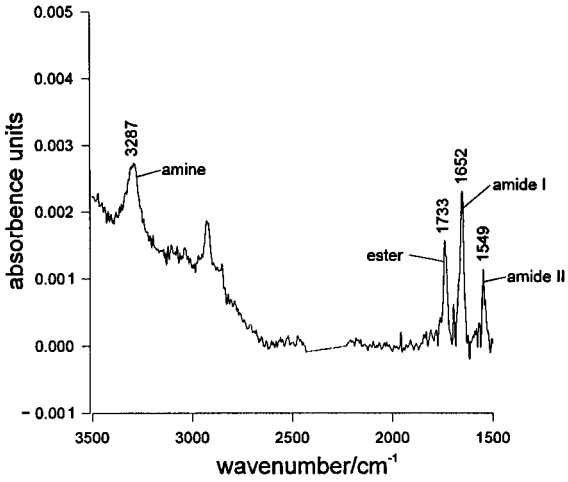

Figure 8. FT-IR spectrum of PBLG polymerized from a wafer with 100% amino groups.

The results of the polymerization with regard to the thickness and the roughness of the polymer layer varied with the concentration of initiator sites on the surface: A minimum concentration was required to obtain a certain thickness and minimum roughness. Beyond a certain concentration, the polymer layer thickness remained constant while its roughness increased. FT-IR measurements revealed, that all grafted polymers are in the α-helical conformation. An interpretation of the change of contact angles after polymerization and a deeper discussion of the roughness values will follow after investigation of the polymer orientation.

Acknowledgment. We thank Wacker Chemitronic GmbH for the donation of the silicon wafers and the Volkswagen-Stiftung as well as BMBF (Grant FI3MPG) for the financial support of this work.

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(38) According to ref 28 every monomeric unit contributes 1.5 Å to the length of the helix.